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A STUDY TO EVALUATE THE ANALYTICAL PARAMETERS OF *SAPTANGA GUGGULU* AND ITS STANDARDIZATION

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ABSTRACT

Introduction: *Saptanga Guggulu* is a well-known drug of Indigenous System of Medicine. *Guggulu* is a gummy resin of the plant. There are different media explained in literature for *Shodhana* of *Guggulu*. According to the media of purification the quality and pharmacological properties of *Guggulu* may vary. In the present analytical study, four batches of *Guggulu* purified by four different media have been used to prepare four batches of *Saptanga Guggulu* and its determination of standardization parameters done **Methods**: The drug *Saptanga Guggulu* prepared by *Guggulu Shodhana* with the four different media i.e. *Triphala Kwatha* (S_1), *Godugdha* (S_2), *Vasapatra Swarasa* (S_3) and *Nirgundipatra Swarasa* along with *Haridra Churna* (S_4) has to undergo certain tests to check if it meets with the quality of the standard preparation. The different tests which the drug underwent are: Organoleptic character, Physico-chemical tests and Chromatographic study **Results:** The results obtained from the analytical study can be used as the pharmaceutical standards for the drug *Saptanga Guggulu*. The various methods of purification used in *Guggulu Shodhana* can also be used as standard for deciding the method of preparation of a drug. **Conclusion**: The chemical constituents identified by analytical study of *Saptanga Guggulu* have potent anti-inflammatory effect either individually or synergistically. The pharmaceutical and analytical study have confirmed the authenticity and quality of the four batches of *Saptanga Guggulu* prepared by *Guggulu Shodhana* in four different media i.e. *Triphala Kwatha, Godugdha, Vasapatra Swarasa, Nirgundipatra Swarasa* along with *Haridra Churna*.

Keywords: *Guggulu*, Analytical Study, Standardization, *Shodhana*

INTRODUCTION

Saptanga Guggulu[1] has been selected which is a *Guggulu Kalpana* mentioned in *Sharangadhara Samhita, Vranashotha Adhikara*. Right from the Vedic period *Guggulu* is a well-known drug of Indigenous System of Medicine. *Guggulu*[2] is a gummy resin of the plant. The formulations made of *Guggulu* come under *Guggulu Kalpana*. To make it fit for internal use, it has to undergo the process of *Shodhana*. There are different media explained in literature for *Shodhana* of *Guggulu*[3]. According to

the media of purification the quality and pharmacological properties of *Guggulu* may vary. Depending on the change in properties the therapeutic effect may also vary. In the present study four batches of *Guggulu* purified by four different media have been used to prepare four batches of *Saptanga Guggulu*. The four batches of *Guggulu* are:*Guggulu Shodhana* in *Triphala Kwatha*, *Guggulu Shodhana* in *Godugdha*, *Guggulu Shodhana* in *Vasapatra Swarasa* and *Guggulu Shodhana* in *Nirgundipatra Swarasa* along with *Haridra Churna*. The *Aushadha Kalpana* are prepared by different pharmaceutical processing techniques applied to the crude drugs to get the desired therapeutic effect. Crude and unprocessed drugs are rarely administered in Ayurveda, there are many formulations that have been described in Ayurveda from simple *Churna* of herbal drugs to complex *Sindoora* and *Bhasma*. They all are called as *Aushadha Kalpana*.

Among all these pharmaceutical processes *Shodhana* is one of them. In our text, for a single drug many methods of *Shodhana* in different ways have been mentioned. In view of the present trend of commercialization in the preparation and marketing of Ayurvedic medicine and to ensure the interests of the profession and public, it has become our prime duty to establish the standard pharmaceutical *Shodhana* process as well as to find out the physico-chemical changes occurring during the process. A standard is a numerical value, which quantify the parameters and thus denotes quality and purity of material. The numeric value expressed in various metric units of measurements actually gives the quantitative value of the parameter, which is used for making the standard.

Methods

In the present context *Guggulu Shodhana* has been done in four batches of *Guggulu* in *Triphala Kwatha, Godugdha, Vasapatra Swarasa* and *Nirgundipatra Swarasa* along with *Haridra Churna* respectively, according to Ayurvedic Formulary of India (2nd revised English edition).

Ingredients of the drug *SAPTANGA GUGGULU*.

The drug *Saptanga Guggulu* taken for the present study has been taken from *Bhaishajya Ratnavali, Vranashotha Adhikara*. The ingredients and ratio of the quantity of the drugs are:

Table 1: The ingredients and the ratio of the quantity of the drugs

Sanskrit name	Botanical name	Family	Part used	Quantity
Vidanga[4]	Embilica ribes Burm. F	Myrsinaceae	Fruit	1 part
Haritaki[5]	Terminalia chebula Retz.	Combrataceae	Fruit	1 part
Vibhitaki[6]	Terminalia bellerica Roxb.	Combrataceae	Fruit	1 part
Amalaki[7]	Embilica officinalis Gaertn.	Euphorbiaceae	Fruit	1 part
Shunti[8]	Zingiber officinale Roscoe.	Zingiberaceae	Rhizome	1 part
Maricha[9]	Piper nigrum Linn.	Piperaceae	Fruit	1 part
Pippali[10]	Piper longum Linn.	Piperaceae	Fruit	1 part
Guggulu[11]	Commiphora mukul (Hook ex. Stocks) Engl.	Burseraceae	Gum	7 parts

Collection of the drugs

All the ingredients required for the preparation of the drug were collected from Alva's Pharmacy, Mijar, Moodabidri.

Three samples of *Guggulu* were collected, 1) 1 kg from Alva's Pharmacy, Mijar, 2) 1 kg from Kottakkal, Malappuram Dist. Kerala and 3) 220g from Udupi Dist. Karnataka.

Preparation of *Saptanga Guggulu*

The drug *Saptanga Guggulu* prepared by *Guggulu Shodhana* with the four different media i.e. *Triphala Kwatha* (S1), *Godugdha* (S_2), *Vasapatra Swarasa* (S_3) and *Nirgundipatra Swarasa* along with *Haridra Churna* (S_4) has to undergo certain tests to check if it meets with the quality of the standard preparation. The different tests which the drug underwent are: A.

Organoleptic character, B. Physico-chemical tests and C. Chromatographic study

A. Organoleptic character[4]

It is done my taking each sample and perceiving the colour, taste, odour and appearance by subjecting the samples with the sensory organs.

B. Physico-chemical tests[5]

- **Loss on drying at 105°C**

10 g of sample was placed in tarred evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample[6].

- **Total Ash**

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample[7].

- **Acid insoluble Ash**:

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ash less filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug[8].

- **Alcohol soluble extractive**

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value[9].

- **Water soluble extractive**:

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value[10].

- **Determination of pH**

Preparation of buffer solutions:

Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH: 1gm of sample was taken and was dissolved in 100ml of distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on for 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result[11].

C. Chromatographic test

- **HPTLC**

One gram of powdered samples were dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. 4µl of each of the above samples were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plates were visualized in UV 254, 366 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. Rf, colour of the spots and densitometric scan were recorded.

Result

A.1. Organoleptic characters

Table 2: showing the organoleptic characters of the four samples of *Saptanga Guggulu*

Organoleptic character	S_1	S_2	S_3	S_4
Colour	Black, Shiny	Light brown	Dark brown	Yellowish brown
Taste	*Kashaya, Katu*	*Katu, Tikta*	*Katu, Tikta*	*Tikta, Katu*
Odour	Predominant smell of *Triphala Kashaya* along the ingredients present in it.	Pleasant smell of the ingredients present in the drug.	Predominant smell of *Vasapatra* along with the other ingredients present in it.	Predominant smell of *Haridra* along with the other ingredients present in it.
Appearance	*Vati*	*Vati*	*Vati*	*Vati*

* S_1 – *Saptanga Guggulu* prepared by *Guggulu Shodhana* with *Triphala Kwatha*, S_2 – *Saptanga Guggulu* prepared by *Guggulu Shodhana* with *Gudugdha*, S_3 – *Saptanga Guggulu* prepared by *Guggulu Shodhana* with *Vasapatra Swarasa*, S_4 – *Saptanga Guggulu* prepared by *Guggulu Shodhana* with *Nirgundipatra Swarasa* and *Haridra Churna*.

A.2. Physiochemical tests

Table 3: Showing the result of standardization parameters of *Saptanga Guggulu* prepared by *Guggulu Shodhana* with 4 different media

Parameters	**Results n=3 %w/w**			
	S1	**S2**	**S3**	**S4**
Loss on drying	16.04	10.79	7.26	4.39
Alcohol soluble extractive value	13.10	18.83	10.60	11.34
Water soluble extractive value	45.25	31.22	34.89	20.74
Total ash	4.38	6.39	6.44	4.78
Acid insoluble ash	0.2	2.10	1.39	3.09
Water soluble ash	2.48	2.19	3.17	3.29
pH	5.0	5.0	5.0	5.0

A.3. Chromatographic studyHPTLC (Fig.3)

Table 4: R_f values of samples at short UV (254nm) (Fig.4, 5, 6, 7)

S1	S2	S3	S4
-	0.07 (L. green)	0.07 (L. green)	0.07 (L. green)
0.11 (L. green)	-	-	-
0.16 (L. green)	0.16 (L. green)	0.16 (L. green)	0.16 (L. green)
0.20 (L. green)	0.20 (L. green)	0.20 (L. green)	-
-	-	-	0.21 (L. green)
0.24 (D. green)	0.24 (D. green)	0.24 (D. green)	0.24 (D. green)
-	0.28 (L. green)	0.28 (L. green)	-
0.31 (L. green)	0.31 (D. green)	0.31 (L. green)	0.31 (L. green)
-	0.33 (L. green)	0.33 (L. green)	0.33 (L. green)
0.36 (L. green)	-	-	-
-	0.40 (D. green)	0.40 (D. green)	0.40 (D. green)
0.43 (D. green)	-	-	-
-	0.58 (L. green)	0.58 (L. green)	0.58 (L. green)
-	0.74 (L. green)	0.74 (L. green)	0.74 (L. green)
-	-	-	0.84 (L. green)

***L-Light, D-Dark**

Table 5: R_f values of samples at long UV (366nm) (Fig.8, 9, 10, 11)

S1	S2	S3	S4
-	-	0.04 (F. blue)	0.04 (F. blue)
0.07 (F. blue)	0.07 (F. blue)	0.07 (F. blue)	-
-	0.11 (F. blue)	0.11 (F. blue)	0.11 (F. blue)
-	-	-	0.13 (F. blue)
0.15 (F. blue)	0.15 (F. blue)	0.15 (F. blue)	0.15 (F. blue)
-	0.20 (F. blue)	0.20 (F. blue)	-
-	0.23 (F. blue)	0.23 (F. blue)	-
0.27 (F. blue)	0.27 (F. blue)	0.27 (F. blue)	0.27 (FD. Yellow)
0.29 (F. blue)	-	-	-
-	0.34 (F. blue)	0.34 (F. blue)	0.34 (F. blue)
0.37 (F. blue)	-	-	-
-	-	0.43 (F. blue)	-
0.47 (F. blue)	0.47 (F. blue)	0.47 (F. red)	-
-	0.52 (F. blue)	0.52 (F. blue)	0.52 (F. blue)
0.57 (F. blue)	0.57 (F. blue)	0.57 (F. blue)	0.57 (F. red)
-	0.61 (F. red)	0.61 (F. red)	0.61 (F. red)
-	0.74 (F. blue)	0.74 (F. blue)	0.74 (F. blue)
0.77 (F. blue)	-	-	-
-	0.85 (F. blue)	0.85 (F. blue)	0.85 (F. blue)
0.89 (F. blue)	-	-	-

*** F-Fluorescence**

Table 6: R_f values of samples after derivatisation (620nm)

S1	S2	S3	S4
-	0.07 (D. purple)	0.07 (L. purple)	0.07 (Purple)
-	0.13 (D. purple)	0.13 (L. purple)	0.13 (Purple)
0.19 (L. purple)	0.19 (D. purple)	-	0.19 (Purple)
0.23 (L. purple)	0.23 (L. purple)	0.23 (L. purple)	-
0.25 (L. purple)	-	0.25 (L. purple)	0.25 (Orange)
0.37 (L. purple)	0.38 (D. purple)	0.37 (L. purple)	0.37 (L. purple)
-	0.40 (L. purple)	0.40 (L. purple)	0.40 (L. purple)
0.43 (L. purple)	-	-	-
0.50 (L. purple)	0.50 (L. purple)	0.50 (L. purple)	0.50 (L. purple)
-	0.54 (L. purple)	0.54 (L. purple)	-
-	-	-	0.55 (L. purple)
0.57 (L. purple)	0.57 (L. purple)	0.57 (L. purple)	-
-	0.64 (L. purple)	-	-
-	0.72 (L. purple)	-	0.72 (L. purple)
-	-	-	0.85 (L. purple)
-	-	-	0.94 (L. purple)
-	0.95 (L. purple)	0.95 (L. purple)	-

***L-Light, D-Dark, F-Fluorescence**

DISCUSSION

Discussion on Organoleptic characters

The colour of the sample S_1 was black and shiny in nature. It might be due to the *Guggulu* used, which was black and shiny in nature after *Shodhana*. The predominant taste was *Kashaya*, which can be attributed to the taste of *Triphala Kwatha* which was used for the *Shodhana* of *Guggulu*. The *Katu Rasa* present in it can be attributed to the predominant taste of *Trikatu* present in it. The odour of *Triphala Kwatha* and also the odour of the ingredients were perceivable while testing.

The colour of the sample S_2 was light brown. It might be due to the *Guggulu* used, which was light brown in colour after *Shodhana*. The predominant taste was *Katu*, which can be attributed to the *Trikatu* present in it, followed by *Tikta* Rasa which was the *Anu Rasa*. The odour of the ingredients was perceivable while testing.

The colour of the sample S_3 was dark brown. It might be due to the *Guggulu* used, which was black in colour after *Shodhana*. After mixing with the other ingredients the colour changed to dark brown. The predominant taste was *Katu*, which can be attributed to the *Trikatu* present in it, followed by *Tikta Rasa*. The odour of *Vasapatra Swarasai* and the ingredients were perceivable while testing.

The colour of the sample S_4 was yellowish brown. It might be due to the addition of *Haridra Churna* along with *Nirgundipatra Swarasa* during the *Shodhana* procedure of *Guggulu*. The predominant taste was *Katu*, which can be attributed to the *Trikatu* present in it, followed by *Tikta Rasa*. The odour of *Haridra* and the ingredients were perceivable while testing.

Discussion on Physiochemical evaluation

1. Loss on drying

This is the method to determine the moisture content of a drug. It aids to prevent the decomposition of the drugs either due to chemical change or microbial contamination. From the results obtained for loss on drying, the samples S_1, S_2, S_3 and S_4 showed 16.04%, 10.79%, 7.26% and 4.39% respectively.

2. Determination of ash value

Ash value is the criterion to judge authenticity and purity of crude drugs. The residue remaining after incineration is the ash content of the drug. These could be inorganic salts such as carbonates, sulphates, phosphates, silicates etc. naturally occurring in the drug or adhered to it or deliberately added to it in order to adulterate the drug. Total ash is to measure the total amount of plant material remaining after ignition of the drug. Acid insoluble ash or water soluble ash content is the residue obtained after boiling the total ash either with dilute hydrochloric acid or water which measures the amount of sand and silica matter present in the drug. The results of ash value for the four samples S_1, S_2, S_3 and S_4 showed total ash content of 4.38%, 6.39%, 6.44% and 4.78% respectively. The acid insoluble ash values of the 4 samples in order were 0.2%, 2.10%, 1.39%, 3.09% respectively. The water soluble ash values of the four samples in order were 2.48%, 2.19%, 3.17% and 3.19% respectively.

3. Determination of extractive value

Extractive value measures the nature of the chemical constituents present in a crude drug. It is essential for the estimation of specific chemical constituents soluble in that particular solvent used for extraction. The results of alcohol soluble extractive values of the four samples in order were 13.10%, 18.83%, 10.60% and 11.34% respectively. Water soluble extractive values of the four samples in order were 45.25%, 31.22%, 34.89% and 20.74% respectively.

4. Determination of pH

The pH represents the acidity or alkalinity of the product. The pH of all the samples S_1, S_2, S_3 and S_4 was 5.0 which show that it was acidic in nature. It indicates a good shelf life of the drug.

Discussion on Chromatography study

This method helps in separation of chemical constituents between two phases: a mobile and a stationary phase. It is an important analytical tool for qualitative and quantitative analysis of the drugs. In this study, thin layer chromatography (TLC) and High Performance Thin Layer Chromatography

(HPTLC) methods were conducted. The plates developed were visualised in UV 254nm, 366nm and then post-derivatised.

Discussion on Limitation of Study

The study can be properly standardised if the tablets are made in pharmaceutical standards as per the required dosage. In this study tablets were rolled by hand with the application of ghee as per classical reference. Standardization parameters on hardness test, uniformity of size and disintegration test can be done once the formulation is prepared pharmaceutically to be prescribed to the public

Discussion on Analytical Study

Data generated by the analytical study of any standard medicine suggest the quality of drug and specific therapeutic effects. If different physical and chemical components of medicine differ from the standard range of values, then therapeutic values of drug will not be the same as standard one. So, for quality control of drug analytical study gives us the valuable data. To make therapeutic effect of a drug predictable and reproducible, which is the basic essence of quality control, analytical values must be the same as to standard. *Saptanga Guggulu* mentioned here is indicated in *Shotha* as per the classical reference[13]

In ancient days, the drugs were prepared by the physicians him, with the help of experienced assistants in their own pharmacies attached to their clinics. Nowadays the trends have entirely changed. The demand of Ayurvedic drugs have increased by many folds and availability of raw materials are also limited. So, there are chances of production of low quality drugs for the commercial benefits.

The quality of final products depends on the raw material used as well as on the pharmaceutical process adopted. The increasing demand for Ayurvedic drugs have made it necessary that some sort of uniformity in the manufacturing of Ayurvedic medicine should be brought out. The need has also been felt for statutory control, to ensure standards of Ayurvedic drugs. Chemical study ensures not only chemical constituents but also suggests us the standards of any preparation. It not only gives standards of the products but indirectly gives suggestions for further advancement if required.

CONCLUSION

As the analytical study of *Saptanga Guggulu* is done for the first time, the values obtained in this study can be used as a reference standard for further studies going to be conducted in the future except for the hardness test, uniformity of size and disintegration test. Thus the pharmaceutical and analytical study has confirmed the authenticity and quality of the four batches of *Saptanga Guggulu* prepared by *Guggulu Shodhana* in four different media i.e. *Triphala Kwatha, Godugdha, Vasapatra Swarasa, Nirgundipatra Swarasa* along with *Haridra Churna*.

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10. Dr Pandey Gyanendra; Dravya guna vijnana (Materia-media-vegetable drugs); Chaukhamba Krishnadas Academy, Varanasi; part III; page 116.

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Fig 2. Four batches of *Saptanga Guggulu*

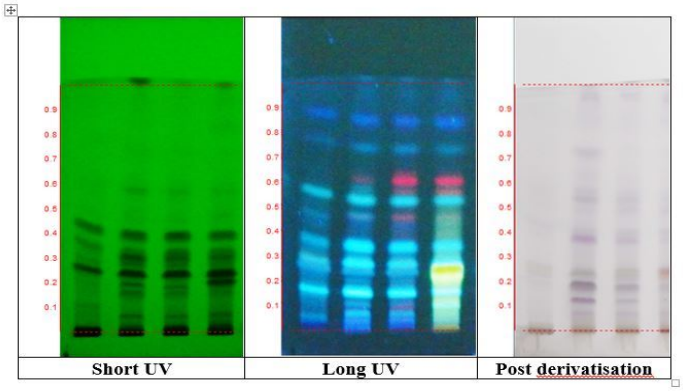

Fig 3. HPTLC photo documentation of ethanol extract of S_1, S_2, S_3, S_4

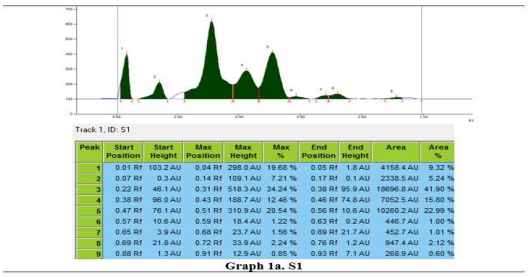

Fig 4. Densitometric scan of the sample S_1 at 254nm

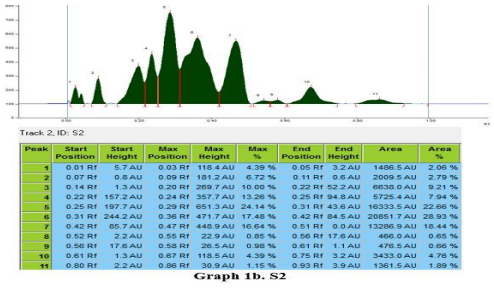

Fig 5. Densitometric scan of the sample S_2 at 254nm

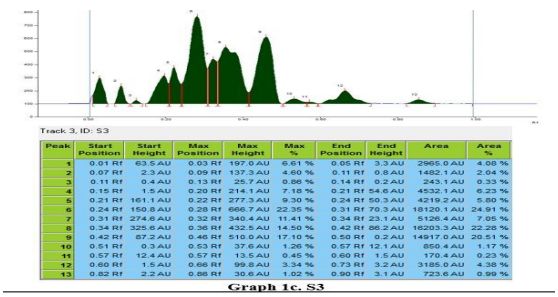

Fig 6. Densitometric scan of the samples S_3 at 254nm

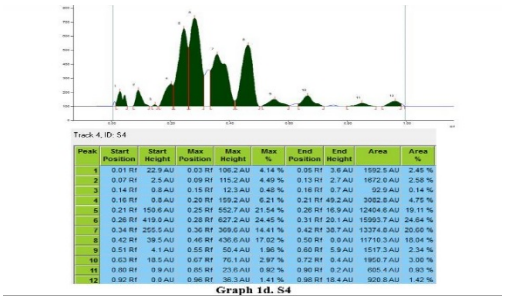

Fig 7. Densitometric scan of the samples S_4 at 254nm

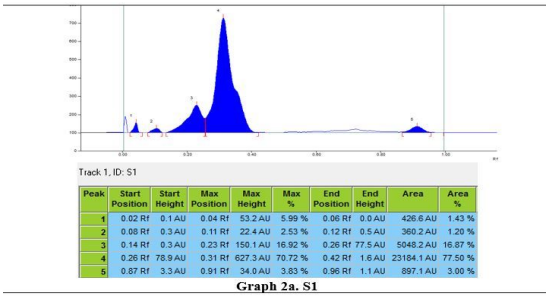

Fig 8. Densitometric scan of the samples S_1 at 366nm

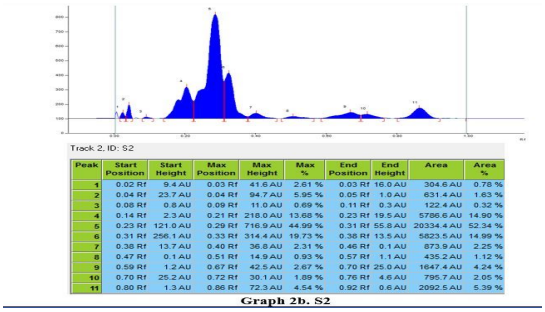

Fig 9. Densitometric scan of the samples S_2 at 366nm

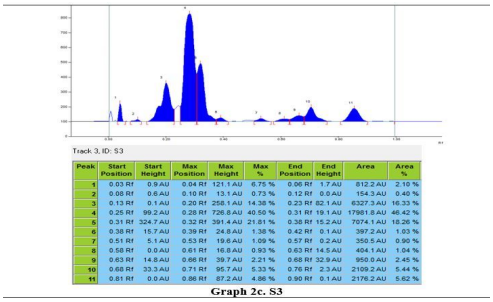

Fig 10. Densitometric scan of the samples S_3 at 366nm

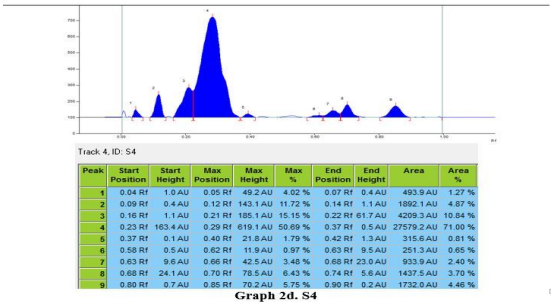

Fig 11**.** Densitometric scan of the samples S_4 at 366nm

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